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(54) Title: METHODS FOR PRODUCING AMYLASE ENZYMES

(57) Abstract

The present invention relates to methods for producing new amylase enzymes using random mutation of DNA encoding for an amylase enzyme followed by isolation of amylase enzyme by evaluating the ability to hydrolyze certain starches in the presence of certain cleaning composition ingredients.

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METHODS FOR PRODUCING AMYLASE ENZYMES

TECHNICAL FIELD

The present invention relates to methods for producing new amylase enzymes using random mutation of DNA encoding for an amylase enzyme followed by isolation of amylase enzyme by evaluating the ability to hydrolyze starches in the presence of select cleaning composition ingredients. The present invention also relates to detergent compositions comprising a new amylase enzyme produced by the above method and cleaning composition ingredients.

BACKGROUND OF THE INVENTION

For a number of years amylase enzymes have been used for a variety of different purposes, the most important of which are starch liquefaction, textile desizing, starch modification in the paper and pulp industry, and for brewing and baking. A further use of amylases, which is becoming increasingly important is the removal of starch-containing soils and stains during the washing of fabrics, hard surfaces and dishes.

Indeed, amylase enzymes have long been recognized in dishwashing, hard surface cleaning and laundry compositions to provide the removal of starchy food residues or starchy films from dishware, flatware, glasses and hard surfaces or to provide cleaning performance on starchy soils as well as other soils typically encountered in laundry applications.

WO 94/02597, Novo Nordisk A/S published February 3, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO 94/18314, Genencor, published August 18, 1994 and WO 95/10603, Novo Nordisk A/S, published April 20, 1995.

Other amylases known for use in cleaning compositions include both α- and β-amylases. α-Amylases are known in the art and include those disclosed in U.S. Patent No. 5,003,257; EP 252,666; WO 91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent Specification No. 1,296,839 (Novo).

Examples of commercial α-amylases products are Termanyl®, Ban®, Fungamyl®, and Duramyl®, all available from Novo Nordisk A/S, Denmark.

Recently, new amylases have been identified and are described in WO 95/26397, Novo Nordisk A/S, published October 5, 1995, disclosing an α-amylase having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25 to 55°C and at a pH value in the range of 8 to 10.

Thus, specific amylase enzymes have already found utility in laundry and cleaning compositions. However, these enzymes still have there limitations when used in certain cleaning composition matrixes. Most product applications create a harsh environment for these enzymes, and common detergent ingredients such as builders and bleaches are likely to inactivate these enzymes. Thus, there continues to be a need to identify and isolate new amylase enzyme variants for use in cleaning compositions.

The above-noted genetically engineered amylases are typically the product of rational design wherein specific sites within known amylase enzymes are targeted for selected modification of the amino acid sequence, either by substituting a different amino acid for the existing one and/or by eliminating one amino acid or a portion of the enzyme chain. Clearly, this approach can produce improved amylase enzymes, as evidenced by the above-noted publications and patents, but progress is slow and screening-intensive. Further, as noted above, these amylase enzymes may provide some benefits in cleaning product applications, but their activity is much reduced relative to their activity in their natural environment.

Several methods for modifying DNA strands are known, including variant generation, mutagenesis, and DNA shuffling. The gene to be improved is mutagenised, recombined, and expressed variants of the gene are selected and/or screened to generate "positive" leads by using an algorithm that is termed a "directed evolution" approach (as described by Moore & Arnold, Nature Biotechnology 14:458-467; 1996). This algorithm is applicable to any enzyme or protein to be changed, provided a suitable screen can be developed, and is described in detail below. Random mutagenesis, the concept of using single-mutant libraries, and the technique of DNA shuffling (recombination) have been described previously, most recently in WO 97/09446, published March 13, 1997 by Novo Nordisk (random mutagenesis which uses a "catcher molecule" that binds specifically with - and then is used to separate - the enzyme variants that exhibit only the desired property).

In terms of variant generation, it is desirable to examine as many different variants as possible, to be assured of getting as many positive "hits" as possible. However, it turns out that the number of amino acid mutations introduced per enzyme is limited by the screenable number of mutants; i.e. screening is limited by the numbers of possible variants. For example, for an average

size enzyme of ~300 amino acids, the number of possible sequences containing a given number of amino acid changes increases exponentially with the increasing number of changes. For example, the number of possible variants containing all possible single amino acid changes is 5.7×10^3, for all possible double changes is $\sim 1.6 \times 10^7$, and for all possible triple changes is $\sim 3.1 \times 10^{10}$. Clearly, one is limited to examining single changes (or possibly double changes if a robotics system is used) if one is to examine all possible changes and be assured the greatest probability of success. This approach of screening libraries (populations) of single-mutant variants has been proposed previously by Moore & Arnold (Nature Biotechnology 14:458-467; 1996) and Arnold (Chem. Eng. Sci. in press; 1996).

Thus, the science of genetic engineering has designed a variety of general methods for modifying DNA coding. However, for purpose of providing amylase enzymes of use in cleaning composition, it is necessary that the method used produce a manageable number of variants and that an efficient and effective screening method (which correctly limits the still vast array of amylase variants produced) be used to produce a reasonable number of amylase enzymes capable of more defined product use and testing.

It is therefore an object to provide a method for producing amylase enzymes useful for cleaning compositions produced by a random DNA mutation using a method involving mutagenesis combined with a selected screening process involving measuring for increased starch hydrolytic activity in the presence of cleaning composition components. The present invention also relates to detergent compositions comprising novel amylases having increased hydrolytic activity and detergent composition ingredients. These and other objects of the present invention will become apparent from the detailed description hereinafter.

BACKGROUND ART

A process for generating random mutations using "error prone" polymerase chain reaction ("error-prone PCR") is described in Leung et al., Technique 1:11-15; 1989, and Cadwell & Joyce, PCR Methods Applic. 2:28-33; 1992. See also: WO 95/22625, published August 24, 1995 by Affymax Technologies N.V.

The "directed evolution" approach to gene mutation is described in Moore & Arnold, Nature Biotechnology 14:458-467; 1996. Screening libraries (populations) of single-mutant variants has been described in Moore & Arnold, Nature Biotechnology 14:458-467; 1996, and Arnold, Chem. Eng. Sci. in press; 1996.

WO 94/02597, Novo Nordisk A/S published February 3, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO 94/18314, Genencor, published

August 18, 1994 and WO 95/10603, Novo Nordisk A/S, published April 20, 1995. Other amylases known for use in cleaning compositions include those disclosed in U.S. Patent No. 5,003,257; EP 252,666; WO 91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent Specification No. 1,296,839 (Novo). Recently, new amylases have been identified and are described in WO 95/26397, Novo Nordisk A/S, published October 5, 1995, disclosing an α-amylase having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25 to 55°C and at a pH value in the range of 8 to 10. WO 97/09446, Novo Nordisk A/S published March 13, 1997, describes using random mutagenesis and a "catcher molecule" for identifying enzymes suitable for use in detergents.

SUMMARY OF THE INVENTION

The present invention relates to a method for producing DNA for new amylase enzymes useful for cleaning applications. This method comprises:

(a) randomly mutating, by chemical and/or enzymatic mean, single or double site mutations in one or more DNA encoding for an amylase enzyme;

(b) incorporating the mutated DNA from step (a) into a microorganism which is capable of expressing amylase enzyme;

(c) separating into individual isolates organisms from step (b) such that a minimum number (preferably on average about one) of the organisms containing mutated DNA is present in each isolate;

(d) producing amylase enzyme from one or more of the isolates;

(e) collecting the mutated DNA from one or more isolates that produce amylase which hydrolyzes starch at a rate of at least about 25% faster than the parent amylase in the presence of cleaning composition ingredients comprising at least surfactants, builders and/or bleaches, preferably at a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1;

(f) optionally, DNA shuffling during a separate step or concurrently with any of steps (a) to (e); and

(g) optionally repeating, one or more times, steps (a) to (f) and then collecting the mutated DNA.

The present invention also relates to a method for producing organisms which produce amylase enzymes useful for cleaning applications. This method comprises:

(a) randomly mutating, by chemical and/or enzymatic mean, single or double site mutations in one or more DNA encoding for an amylase enzyme;

(b) incorporating the mutated DNA from step (a) into a microorganism which is capable of expressing amylase enzyme;

(c) separating into individual isolates organisms from step (b) such that a minimum number (preferably on average about one) of the organisms containing mutated DNA is present in each isolate;

(d) producing amylase enzyme from one or more of the isolates;

(e) collecting the organisms containing mutated DNA from one or more isolates that produce amylase which hydrolyzes starch at a rate of at least about 25% faster than the parent amylase in the presence of cleaning composition ingredients comprising at least surfactants, builders and/or bleaches, preferably at a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1;

(f) optionally, DNA shuffling during a separate step or concurrently with any of steps (a) to (e); and

(g) optionally repeating, one or more times, steps (a) to (f) and then collecting the organisms containing mutated DNA that produce the amylase.

The present invention also relates to detergent compositions comprising:

(a) from about 0.0001% to about 2% by weight of an amylase enzyme, having a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1, produced by an organism containing mutated DNA prepared by a method according to the present invention; and

(b) from about 98% to about 99.9999% of cleaning composition ingredients comprising one or more of surfactants, builders, and bleaching agents.

All parts, percentages and ratios used herein are expressed as percent weight unless otherwise specified. All documents cited are, in relevant part, incorporated herein by reference.

DETAILED DESCRIPTION OF THE INVENTION

The present invention method comprises the following steps, which are described in detail hereinafter.

(A) Random Mutation Step:

The process of generating random mutations in genes for the present invention methods is accomplished by any method which randomly produces only one or two changes (on average) per DNA strand. A preferred process of generating such random mutations is using "error prone" polymerase chain reaction ("error-prone PCR") as described in detail by Leung et al. (Technique

1:11-15; 1989) and Cadwell & Joyce (PCR Methods Applic. 2:28-33; 1992). The gene or gene fragment of interest is excised from a plasmid vector that has been engineered to contain the appropriate restriction enzyme site. The fragment is mutagenized by error-prone PCR, and the population of mutant DNA molecules restricted with the appropriate enzymes and ligated back into the original plasmid vector to generate a mutant "library", for example as shown in the Leung et al. article

The overall error rate of the "error-prone" PCR process must be adjusted so that the average number of mutations in the population at the amino acid (protein) level is one. Because of the redundancy of the genetic code, this translates to an average of approximately two mutations at the DNA level in the variant gene library. The error rate may be adjusted, for example, by altering the manganese concentration in the PCR reaction (see, for example, the reaction conditions described in the Cadwell & Joyce article noted above). This must be done for each gene to be mutated at the beginning of the process. Additional details regarding the use of error-prone PCR are found in WO 95/22625, published August 24, 1995 by Affymax Technologies N.V., incorporated by reference herein in its entirety.

The DNA selected for this random mutation step may be any DNA which encodes for an α -amylase protein. However, it is preferred herein to use the DNA which encodes for known α-amylases which have been evaluated for use in cleaning compositions. Such amylase enzymes include those described in WO 95/26397.

Specific DNA encoding for amylase enzymes useful in detergent compositions therefore include:

(a) α-amylases characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas® α-amylase activity assay, as described in detail in WO 95/26397.

(b) α-amylases according to (a) comprising the amino sequence shown in SEQ ID No. 1 or an α-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1.

A peptide is considered to be X% homologous to the parent amylase if a comparison of the respective amino acid sequences, performed via algorithms, such as the one described by Lipman and Pearson in Science 227, 1985, p. 1435, reveals an identity of X%.

(c) α-amylases according to (a or b) wherein the α-amylase is obtainable from an alkalophilic Bacillus species; and in particular, from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935.

In the context of the present invention, the term "obtainable from" is intended not only to indicate an amylase produced by a Bacillus strain but also an amylase encoded by a DNA sequence isolated from such a Bacillus strain and produced in a host organism transformed with said DNA sequence.

(d) Amylases showing positive immunological cross-reactivity with antibodies raised against an α-amylase having an amino acid sequence corresponding to SEQ ID No. 1, or Termamyl.

(e) Variants of the following parent α-amylases which (i) have the amino acid sequence shown in SEQ ID No. 1, or (ii) displays at least 60 %, preferably 80%, homology with this amino acid sequence, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having this amino acid sequence, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α-amylase having this amino acid sequence; in which variants:

1. at least one amino acid residue of said parent α-amylase has been deleted; and/or

2. at least one amino acid residue of said parent α-amylase has been replaced by a different amino acid residue; and/or

3. at least one amino acid residue has been inserted relative to said parent α-amylase;

said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability, increased stability towards oxidation, reduced Ca ion dependency, increased stability and/or α-amylolytic activity at neutral to relatively high pH values, increased α-amylolytic activity at relatively high temperature and increase or decrease of the isoelectric point (pI) so as to better match the pI value for α-amylase variant to the pH of the medium.

(B) Incorporation of Mutated DNA into Microorganisms Capable of Expressing Amylase

Before transformation into organisms capable of expressing amylase (preferred being a Bacillus species), it is highly preferred that the population of mutated DNA molecules (as described herein before, averaging about 1 amino acid change in the final protein product), cloned in an appropriate plasmid DNA vector, be introduced into a standard, commercially available *E. coli* strain (such as XL-2 Blue from Stratagene), using a standard DNA transformation method, followed by isolation of resulting transformants on agar plates (all standard methods). The transformation method is such that each isolated colony that grows on the agar plate has resulted from a single, transformed cell containing a single, mutated amylase gene DNA sequence. These colonies are pooled together and the plasmid DNAs they contain are isolated, to give a population

of plasmid DNA molecules representative of the entire orginal (from the random mutation step hereinbefore) population of mutated DNA molecules.

The purpose of this preferred step is to amplify the amount of the population of the original, mutated DNA molecules, since transformation directly into *Bacillus* species is generally difficult with small amounts of DNA (thus it is preferred to use this intermediate *E. coli* step as an amplification step; no amylase is produced at this stage since *E. coli* is not capable of producing it). Thus, preferably following this amplification process step, the mutated DNA molecules from the random mutation step are introduced into an organism capable of producing amylase enzymes (preferably for the present process a *Bacillus* species) using a standard DNA transformation method.

The cells useful in this invention may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, e.g., a bacterial or a fungal (including yeast) cell.

Examples of suitable bacteria are gram positive bacteria such as Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coaqulans, Bacillus circulans, Bacillus lautus, Bacillus megaterium, Bacillus thuringiensis, Streptomyces lividans or Streptomyces murinus, or gram negative bacteria such as E. coli.

The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known per se.

The yeast organism may favorably be selected from a species of Saccharomyces or Schizosaccharomyces, e.g., Saccharomyces cerevisiae. The filamentous fungus may advantageously belong to a species of Aspergillus, e.g., Aspergillus oryzae or Aspergillus niger. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. A suitable procedure for transformation of Aspergillus host cells is described in EP 238,023.

Preferred α-amylases of the invention are obtainable from an alkaliphilic Bacillus species, particularly from one of the Bacillus strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 9375. In the context of the present invention, the term "obtainable from" is intended not only to indicate an α-amylase produced by a Bacillus strain but also an α-amylase encoded by a DNA sequence isolated from such a Bacillus strain, modified by the present invention process, and produced in a host organism transformed with this mutated DNA sequence.

The strain NCIB 12289 is described in detail in EP 277,216. The strain NCIB 12289 has been deposited according to the Budapest Treaty on the International Recognition of the Deposits

of Microorganisms for the Purpose of Patent Procedures, on July 8, 1996 at The National Collection of Industrial Bacteria (NCIB) under accession No. NCIB 12289.

The strain NCIB 12512 is described in detail in EP 277,216. The strain NCIB 12512 has been deposited according to the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purpose of Patent Procedures, on August 5, 1987 at The National Collection of Industrial Bacteria (NCI) under accession No. NCIB 12512.

The strain NCIB 12513 is described in detail in EP 277,216. The strain NCIB 12513 has been deposited according to the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purpose of Patent Procedures, on August 5, 1987 at The National Collection of Industrial Bacteria (NCIB) under accession No. NCIB 12513.

The strain DSM 9375 has been deposited according to the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purpose of Patent Procedures, on August 16, 1994 at Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) under Accession No. DSM 9375.

According to the invention, an α-amylase-encoding DNA sequence produced by methods described above, can be expressed, in enzyme form, using an expression vector which typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

The recombinant expression vector carrying the DNA sequence encoding an α-amylase produced by the present invention method may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extra chromosomal entity, the replication of which is independent of chormosomal replication, e.g., a plasmid, a bacteriophage or an extra chromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an α-amylase, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the

Bacillus licheniformis α-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus Amyloliquefaciens α-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral α-amylase, A. niger acid stable α-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

The expression vector may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the desired α-amylase. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

The vector may also comprise a selectable marker, e.g., a gene the product of which complements a defect in the host cell such as the dal genes from B. subtilis or B. licheniformis, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracyclin resistance. Furthermore, the vector may comprise Aspergillus selection markers such as amdS, argB, niaD and sC, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, e.g., as described in WO 91/17243.

While intracellular expression may be advantageous in some respects, e.g., when using certain bacteria as host cells, it is generally preferred that the expression is extracellular.

Procedures suitable for constructing vectors useful for the present invention process encoding the desired α-amylase and containing the promoter, terminator, and other elements, respectively, are well known to persons skilled in the art (cf., for instance, Sambrook et al. in Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989).

(C) Separation of Microorganisms into Isolates and Amylase Production Steps

The resulting transformants from the proceeding steps, containing the large amount of DNA produced by the random mutation step herein above, and which has been transformed (using standard methods, for example those described in "Biology of Bacilli: Applications to Industry", 1992, R.H. Doi and M. McGloughlin, Editors, Butterworth-Heinemann, Stoneham, MA., pp. 357-359) into the appropriate species (e.g., preferably *Bacillus* species), are isolated on agar plates using standard methods. At this stage, for example, the individual Bacillus colonies that have

derived from single, transformed cells containing single, mutated amylase gene DNA sequences, are able to express the amylase gene (i.e. make variant amylase proteins).

Individual colonies (~10,000) are picked from the agar plates and inoculated into individual wells of plastic, 96-well microtiter plates that each contain a growth medium that results in amylase production (e.g. 100-200 microliters volume). The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the α-amylase of the invention. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g., as described in catalogues of the American Type Culture Collection).

These plates are incubated for 16 hours at the optimal growth temperature of the *Bacillus* species being used, during which time the inoculated colonies grow and (if able to) secrete amylase into the growth medium. The cells are then removed (centrifugation of the plates), and a portion of the supernatants, each potentially containing a different amylase variant, are assayed as described herein after. The α-amylase secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

(D) Amylase Screening

The present invention process produces an α-amylase having an activity at least 25% higher (preferably at least 35% higher, more preferably at least 50% higher, and most preferably at least 75% higher) than the activity of the parent amylase from which the DNA was obtained and mutated by the random mutation step of this process (preferably at a temperature in the range of 25°C to 55°C). Further preferred are amylases produced by the present methods which not only have a specific activity at least 25% greater than the parent amylase from which the DNA was obtained from the random mutation step, but further this amylase has a specific activity at least 25% greater than the commercially available amylase Teramamyl®. These increased activities are as measured in the presence of cleaning composition ingredients selected preferably from surfactants, builders and/or bleaching agents, as described in detail hereinafter.

α-Amylase activity for comparison versus the parent amylase is preferably determined by a method employing Phadebas® tablets as substrate. Phadebas tablets (Phadebas® Amylase Test, supplied by Pharmacia Diagnostic) contain a cross-linked insoluble blue-colored starch polymer

which has been mixed with bovine serum albumin and a buffer substance and tableted. One Phadebas tablet is added to 6 ml of detergent solution and vortexed for 15 seconds.

180 µl of the Phadebas suspension containing cleaning composition ingredients, comprising at least surfactant and/or builder and/or bleaching agents as described in detail herein after, is added to each well in a 96-well plate. [It is preferred that the suspension contain a fully formulated detergent composition, comprising surfactant and builder, and also preferably one or more ingredients selected from bleaching agents, detergent polymers, and/or chelants, as described in greater detail hereinafter.] To each well, 20 µl of enzyme which is the supernatant from the cell growth described herein before is added and incubated for 15 min. 1 ml of NaOH is added after 15 min of incubation and vortexed for 15 sec. The reaction solution is then filtered. The starch, which is hydrolyzed by the alpha-amylase, gives soluble blue fragments and the absorbance of the resulting blue solution is measured spectrophotometrically at 620 nm. It is important that the measured absorbance is at 620 nm range in the 0.2-2.0 absorbance units to maintain the linearity between activity and absorbance. The reaction solutions must be diluted to have absorbance in this region. Higher absorbance readings is indicative of higher amylolytic activity under the specified conditions of the reaction.

Alternatively, kinetic assay can be performed instead of taking measurements at a given time by measuring absorbances at several time intervals. The above protocol must be followed for each time interval. The slope of the curve of the new libraries vs. the slope of the parent amylase is compared to assess increased amylolytic activity.

A specific procedure to screen the improved amylase in a Loprodyne "Silent Monitor" 96-well assay plates (Pall Biosupport) for development in granular detergents is described as follows. A wash solution is prepared in 6 gpg hardness (3:1 $CaCl_2$:$MgCl_2$) with 0.07 ppm of an amylase with Sequence No. 1 and 1 g/L of the following detergent composition.

	ppm in wash solution	% in composition
I. Bleach		
1. Nonanoyloxybenzene sulfonate	16	2.4
2. Perborate	20	3.1
II. Builders		
1. Na Carbonate	153	23
2. Alumino silicate	287	44
3. Sodium silicate	6	0.9
III. Surfactants:		

1. Linear C 12 alkyl benzene sulfonate	74	11
2. C12-C14 sodium alkyl sulfate condense with an average of 6 ethylene oxides per mol	13	2.0
3. NaC14-15 alkyl sulphate	90	13.6
TOTAL	659	100

The pH of above solution is adjusted to approximately 10.5. 5 mls of this solution is added to 20 mls of de-ionized water. The Phadebas solution is prepared by adding 1 Phadebas tablet in 5 mls of de-ionized water. Phadebas tablets are from Pharmacia and Upjohn (manufactured in Sweden, distributed by Pharmacia Diagnostics Div. of Electro-Nucleonics, Inc. Fairfield, New Jersey). After mixing the Phadebas solution, 143 µl is added into each well of the 96 well plate. To each well, containing the Phadebas solution, 29 µl of diluted wash solution is added. The reaction is progressed for 20 minutes and stopped via addition of 29 µl 1M NaOH per well. The contents of the loprodyne plate is filtered using a Pall vacuum manifold into a clean 96-well Dynatech plate. The concentration of liberated dye in the filtrate is read at 620 nm.

An amylase of this invention delivers at least 25% increased Phadebas hydrolysis versus the amylase having SEQ. ID No. 1 dosed at 0.07 ppm protein at 25°C in the presence of the cleaning composition ingredients.

(E) Mutated DNA Collection:

The DNA sequence encoding an α-amylase produced by the present invention process may be isolated from any cell or microorganism producing the α-amylase in question, using various methods well known in the art. Preferably, cells from wells on the 96-well plates that generated supernatants having a positive result (by demonstrating improved amylolytic activity as described hereinbefore) are then isolated and, preferably re-screened (i.e., inoculation into 96-well plates; growth; re-assay) to confirm that the variant amylases they are producing are indeed improved. These cells from individual wells on the 96-well plated are then grown up (separately) in a larger scale, and the plasmid DNA isolated from them using standard methods. These DNAs (each DNA preparation represents a single amylase variant) can then be used in subsequent rounds of mutagenesis and screening (preferably, only one or two of the DNAs representing the best results are used as the starting points for the next cycle of mutagenesis).

Alternatively, these positive DNAs (usually only 4 or 5) can be recombined by DNA shuffling (as described herein after) to make all possible combinations of the positive mutations.

After DNA shuffling, the resultant combinations are introduced into E.coli, then *Bacillus*, and the resultant cells screened to potentially identify "better positives", in the same way as described hereinbefore. The best positive variant from the DNA shuffling step can then either be taken as the end result, or itself used as the starting point for the next cycle of mutagenesis (with or without shuffling).

(F) Optional DNA Shuffling:

DNA shuffling is a method for recombining unique, but homologeous DNA sequences, whereby all possible combinations of each unique sequence can be generated. In essence, the sequences of interest (e.g., gene fragments, as described above) are enzymatically fragmented, and the fragments used in an assembly PCR reaction (where single-stranded pieces of different variants can prime each other) to reassemble combinations of the individual mutations. In effect what results is a massively parallel reaction, where all possible combinations of the individual positive mutations are put together, and it is this pool of recombinants that, when cloned into the appropriate plasmid vector (same one as described above) constitutes the "shuffled library". The method has been described in detail in WO 95/22625. While DNA shuffling is optional, it is a highly preferred optional part of the present invention process, since it allows all possible combinations of positive variants isolated from screening of mutagenic libraries to be constructed rapidly, and tested.

The above steps may be repeated one or more times to produce further mutated DNA and/or organisms as desired which produce amylase enzyme.

Cleaning Composition Ingredients and Detergent Compositions

The detergent compositions of the invention contain cleaning composition ingedients as described hereinafter. The precise nature of these components, and levels of incorporation thereof will depend on the physical form of the composition, and the nature of the cleaning operation for which it is to be used.

The detergent compositions according to the invention can be liquid, paste, gels, bars, tablets, powder or granular forms. Granular compositions can also be in "compact" form, the liquid compositions can also be in a "concentrated" form.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics, rinse added fabric softener compositions. Pre-or post treatment of fabric include gel, spray and liquid fabric conditioning compositions.

When formulated as compositions suitable for use in a laundry machine washing method, the compositions of the invention preferably contain both a surfactant and a builder compound and additionally one or more detergent components preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional detergent components.

The compositions of the invention can also be used as detergent additive products. Such additive products are intended to supplement or boost the performance of conventional detergent compositions.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 600 to 950 g/litre of composition measured at 20°C.

The "compact" form of the compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition.

In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition.

The inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides.

A preferred filler salt is sodium sulphate.

Liquid detergent compositions according to the present invention can also be in a "concentrated form", in such case, the liquid detergent compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents.

Typically the water content of the concentrated liquid detergent is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the detergent composition.

Surfactants

Preferably, the detergent compositions according to the present invention comprise a surfactant or surfactant system wherein the surfactant can be selected from nonionic and/or anionic and/or cationic surfactants and/or ampholytic and/or zwitterionic and/or semi-polar nonionic surfactants.

The surfactant is typically present at a level of from about 0.1%, more preferably about 1% by weight of the deteregent compositions to about 60%, more preferably about 35%, most preferably 30% about by weight of the detergent compositions.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Examples of suitable nonionic, anionic, cationic, ampholytic, zwitterionic and semi-polar nonionic surfactants are disclosed in U.S. Patent Nos. 5,707,950 and 5,576,282.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula:

$$R^2 - C(O) - N(R^1) - Z,$$

wherein R^1 is H, or R^1 is C_{1-4} hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R^2 is C_{5-31} hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R^1 is methyl, R^2 is a straight C_{11-15} alkyl or C_{16-18} alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants hereof are water soluble salts or acids of the formula $RO(A)_mSO3M$ wherein R is an unsubstituted C_{10}-C_{24} alkyl or hydroxyalkyl group having a C_{10}-C_{24} alkyl component, preferably a C_{12}-C_{20} alkyl or hydroxyalkyl, more preferably C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein.

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1%, more preferably about 3% by weight of such such anionic surfactants to about 40%, more preferably about 20% by weight of such anionic surfactants.

Highly preferred cationic surfactants are the water-soluble quaternary ammonium compounds useful in the present composition having the formula :

$$R_1R_2R_3R_4N^+X^-$$

wherein R_1 is C_8-C_{16} alkyl, each of R_2, R_3 and R_4 is independently C_1-C_4 alkyl, C_1-C_4 hydroxy alkyl, benzyl, and $-(C_2H_{40})_xH$ where x has a value from 2 to 5, and X is an anion. Not more than one of R_2, R_3 or R_4 should be benzyl.

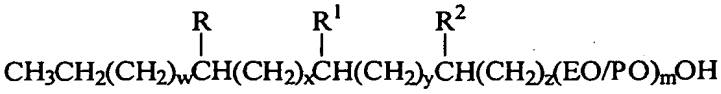
When included therein, the detergent compositions of the present invention typically comprise from about 0.2%, more preferably about 1% by weight of such cationic surfactants to about 25%, more preferably about 8% by weight of such cationic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from about 0.2%, more preferably about 1% by weight of such ampholytic surfactants to about 15%, more preferably about 10% by weight of such ampholytic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from about 0.2%, more preferably about 1% by weight of such zwitterionic surfactants to about 15%, more preferably about 10% by weight of such zwitterionic surfactants.

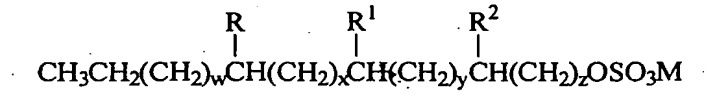
When included therein, the detergent compositions of the present invention typically comprise from about 0.2%, more preferably 1% by weight of such semi-polar nonionic surfactants to about 15%, more preferably about 10% by weight of such semi-polar nonionic surfactants.

The detergent compositions of the present invention can also comprise from about 0.001% to about 100% of one or more (preferably a mixture of two or more) mid-chain branched surfactants, preferably mid-chain branched alkyl alkoxy alcohols having the formula:



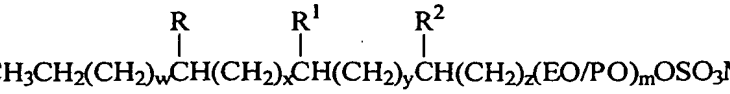
$$CH_3CH_2(CH_2)_wCH(CH_2)_xCH(CH_2)_yCH(CH_2)_z(EO/PO)_mOH$$

mid-chain branched alkyl sulfates having the formula:



$$CH_3CH_2(CH_2)_wCH(CH_2)_xCH(CH_2)_yCH(CH_2)_zOSO_3M$$

and mid-chain branched alkyl alkoxy sulfates having the formula:



$$CH_3CH_2(CH_2)_wCH(CH_2)_xCH(CH_2)_yCH(CH_2)_z(EO/PO)_mOSO_3M$$

wherein the total number of carbon atoms in the branched primary alkyl moiety of these formulae (including the R, R^1, and R^2 branching, but not including the carbon atoms which comprise any EO/PO alkoxy moiety) is from 14 to 20, and wherein further for this surfactant mixture the average total number of carbon atoms in the branched primary alkyl moieties having the above formula is within the range of greater than 14.5 to about 17.5 (preferably from about 15 to about 17); R, R^1, and R^2 are each independently selected from hydrogen, C_1-C_3 alkyl, and

mixtures thereof, preferably methyl; provided R, R^1, and R^2 are not all hydrogen and, when z is 1, at least R or R^1 is not hydrogen. M is a water soluble cation and may comprises more than one type of cation, for example, a mixture of sodium and potassium. The index w is an integer from 0 to 13; x is an integer from 0 to 13; y is an integer from 0 to 13; z is an integer of at least 1; provided w + x + y + z is from 8 to 14. EO and PO represent ethyleneoxy units and propyleneoxy units having the formula:

$$\begin{array}{c}CH_3\\|\\-\!\!-CHCH_2O-\!\!-\end{array} \quad \text{or} \quad \begin{array}{c}CH_3\\|\\-\!\!-CH_2CHO-\!\!-\end{array}$$

respectively, however, other alkoxy units inter alia 1,3-propyleneoxy, butoxy, and mixtures thereof are suitable as alkoxy units appended to the mid-chain branched alkyl moieties.

The mid-chain branched surfactants are preferably mixtures which comprise a surfactant system. Therefore, when the surfactant system comprises an alkoxylated surfactant, the index m indicates the average degree of alkoxylation within the mixture of surfactants. As such, the index m is at least about 0.01, preferably within the range of from about 0.1, more preferably from about 0.5, most preferably from about 1 to about 30, preferably to about 10, more preferably to about 5. When considering a mid-chain branched surfactant system which comprises only alkoxylated surfactants, the value of the index m represents a distribution of the average degree of alkoxylation corresponding to m, or it may be a single specific chain with alkoxylation (e.g., ethoxylation and/or propoxylation) of exactly the number of units corresponding to m.

The preferred mid-chain branched surfactants of the present invention which are suitable for use in the surfactant systems of the present invention have the formula:

$$CH_3(CH_2)_aCH(CH_3)(CH_2)_bCH_2(EO/PO)_mOSO_3M$$

or the formula:

$$CH_3(CH_2)_dCH(CH_3)(CH_2)_eCHCH_2(EO/PO)_mOSO_3M$$

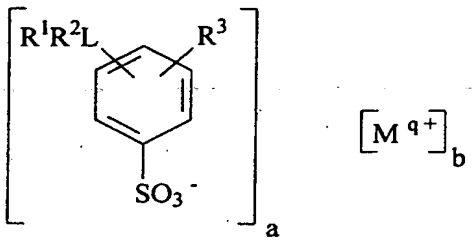
wherein a, b, d, and e are integers such that a + b is from 10 to 16 and d + e is from 8 to 14; M is selected from sodium, potassium, magnesium, ammonium and substituted ammonium, and mixtures thereof.

The surfactant systems of the present invention which comprise mid-chain branched surfactants are preferably formulated in two embodiments. A first preferred embodiment comprises mid-chain branched surfactants which are formed from a feedstock which comprises 25% or less of mid-chain branched alkyl units. Therefore, prior to admixture with any other

conventional surfactants, the mid-chain branched surfactant component will comprise 25% or less of surfactant molecules which are non-linear surfactants.

A second preferred embodiment comprises mid-chain branched surfactants which are formed from a feedstock which comprises from about 25% to about 70% of mid-chain branched alkyl units. Therefore, prior to admixture with any other conventional surfactants, the mid-chain branched surfactant component will comprise from about 25% to about 70% surfactant molecules which are non-linear surfactants.

The surfactant systems of the laundry detergent compositions of the present invention can also comprise from about 0.001%, preferably from about 1%, more preferably from about 5%, most preferably from about 10% to about 100%, preferably to about 60%, more preferably to about 30% by weight, of the surfactant system, of one or more (preferably a mixture of two or more) mid-chain branched alkyl arylsulfonate surfactants, preferably surfactants wherein the aryl unit is a benzene ring having the formula:



wherein L is an acyclic hydrocarbyl moiety comprising from 6 to 18 carbon atoms; R^1, R^2, and R^3 are each independently hydrogen or C_1-C_3 alkyl, provided R^1 and R^2 are not attached at the terminus of the L unit; M is a water soluble cation having charge q wherein a and b are taken together to satisfy charge neutrality.

Bleaching System

The compositions of the present invention preferably comprise a bleaching system. Bleaching systems typically comprise a "bleaching agent" (source of hydrogen peroxide) and an "initiator" or "catalyst". When present, bleaching agents will typically be at levels of from about 1%, preferably from about 5% to about 30%, preferably to about 20% by weight of the composition. If present, the amount of bleach activator will typically be from about 0.1%, preferably from about 0.5% to about 60%, preferably to about 40% by weight, of the bleaching composition comprising the bleaching agent-plus-bleach activator.

Bleaching Agents - Hydrogen peroxide sources are described in detail in the herein incorporated Kirk Othmer's Encyclopedia of Chemical Technology, 4th Ed (1992, John Wiley &

Sons), Vol. 4, pp. 271-300 "Bleaching Agents (Survey)", and include the various forms of sodium perborate and sodium percarbonate, including various coated and modified forms.

The preferred source of hydrogen peroxide used herein can be any convenient source, including hydrogen peroxide itself. For example, perborate, e.g., sodium perborate (any hydrate but preferably the mono- or tetra-hydrate), sodium carbonate peroxyhydrate or equivalent percarbonate salts, sodium pyrophosphate peroxyhydrate, urea peroxyhydrate, or sodium peroxide can be used herein. Also useful are sources of available oxygen such as persulfate bleach (e.g., OXONE, manufactured by DuPont). Sodium perborate monohydrate and sodium percarbonate are particularly preferred. Mixtures of any convenient hydrogen peroxide sources can also be used.

A preferred percarbonate bleach comprises dry particles having an average particle size in the range from about 500 micrometers to about 1,000 micrometers, not more than about 10% by weight of said particles being smaller than about 200 micrometers and not more than about 10% by weight of said particles being larger than about 1,250 micrometers. Optionally, the percarbonate can be coated with a silicate, borate or water-soluble surfactants. Percarbonate is available from various commercial sources such as FMC, Solvay and Tokai Denka.

Compositions of the present invention may also comprise as the bleaching agent a chlorine-type bleaching material. Such agents are well known in the art, and include for example sodium dichloroisocyanurate ("NaDCC"). However, chlorine-type bleaches are less preferred for compositions which comprise enzymes.

(a) Bleach Activators - Preferably, the peroxygen bleach component in the composition is formulated with an activator (peracid precursor). The activator is present at levels of from about 0.01%, preferably from about 0.5%, more preferably from about 1% to about 15%, preferably to about 10%, more preferably to about 8%, by weight of the composition. Preferred activators are selected from the group consisting of tetraacetyl ethylene diamine (TAED), benzoylcaprolactam (BzCL), 4-nitrobenzoylcaprolactam, 3-chlorobenzoylcaprolactam, benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), phenyl benzoate (PhBz), decanoyloxybenzenesulphonate (C_{10}-OBS), benzoylvalerolactam (BZVL), octanoyloxybenzenesulphonate (C_8-OBS), perhydrolyzable esters and mixtures thereof, most preferably benzoylcaprolactam and benzoylvalerolactam. Particularly preferred bleach activators in the pH range from about 8 to about 9.5 are those selected having an OBS or VL leaving group.

Preferred hydrophobic bleach activators include, but are not limited to, nonanoyloxybenzenesulphonate (NOBS), 4-[N-(nonaoyl) amino hexanoyloxy]-benzene

sulfonate sodium salt (NACA-OBS) an example of which is described in U.S. Patent No. 5,523,434, lauryloxybenzenesulphonate (LOBS or C_{12}-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C_{11}-OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA).

Preferred bleach activators are those described in U.S. 5,698,504 Christie et al., issued December 16, 1997; U.S. 5,695,679 Christie et al. issued December 9, 1997; U.S. 5,686,401 Willey et al., issued November 11, 1997; U.S. 5,686,014 Hartshorn et al., issued November 11, 1997; U.S. 5,405,412 Willey et al., issued April 11, 1995; U.S. 5,405,413 Willey et al., issued April 11, 1995; U.S. 5,130,045 Mitchel et al., issued July 14, 1992; and U.S. 4,412,934 Chung et al., issued November 1, 1983, and copending patent applications U. S. Serial Nos. 08/709,072, 08/064,564, all of which are incorporated herein by reference.

The mole ratio of peroxygen bleaching compound (as AvO) to bleach activator in the present invention generally ranges from at least 1:1, preferably from about 20:1, more preferably from about 10:1 to about 1:1, preferably to about 3:1.

Quaternary substituted bleach activators may also be included. The present detergent compositions preferably comprise a quaternary substituted bleach activator (QSBA) or a quaternary substituted peracid (QSP); more preferably, the former. Preferred QSBA structures are further described in U.S. 5,686,015 Willey et al., issued November 11, 1997; U.S. 5,654,421 Taylor et al., issued August 5, 1997; U.S. 5,460,747 Gosselink et al., issued October 24, 1995; U.S. 5,584,888 Miracle et al., issued December 17, 1996; and U.S. 5,578,136 Taylor et al., issued November 26, 1996; all of which are incorporated herein by reference.

Highly preferred bleach activators useful herein are amide-substituted as described in U.S. 5,698,504, U.S. 5,695,679, and U.S. 5,686,014 each of which are cited herein above. Preferred examples of such bleach activators include: (6-octanamidocaproyl) oxybenzenesulfonate, (6-nonanamidocaproyl)oxybenzenesulfonate, (6-decanamidocaproyl)oxybenzenesulfonate and mixtures thereof.

Other useful activators, disclosed in U.S. 5,698,504, U.S. 5,695,679, U.S. 5,686,014 each of which is cited herein above and U.S. 4,966,723Hodge et al., issued October 30, 1990, include benzoxazin-type activators, such as a C_6H_4 ring to which is fused in the 1,2-positions a moiety --C(O)OC(R^1)=N-.

Depending on the activator and precise application, good bleaching results can be obtained from bleaching systems having with in-use pH of from about 6 to about 13, preferably from about 9.0 to about 10.5. Typically, for example, activators with electron-

withdrawing moieties are used for near-neutral or sub-neutral pH ranges. Alkalis and buffering agents can be used to secure such pH.

Acyl lactam activators, as described in U.S. 5,698,504, U.S. 5,695,679 and U.S. 5,686,014, each of which is cited herein above, are very useful herein, especially the acyl caprolactams (see for example WO 94-28102 A) and acyl valerolactams (see U.S. 5,503,639 Willey et al., issued April 2, 1996 incorporated herein by reference).

(b) Organic Peroxides, especially Diacyl Peroxides - These are extensively illustrated in Kirk Othmer, Encyclopedia of Chemical Technology, Vol. 17, John Wiley and Sons, 1982 at pages 27-90 and especially at pages 63-72, all incorporated herein by reference. If a diacyl peroxide is used, it will preferably be one which exerts minimal adverse impact on spotting/filming.

(c) Metal-containing Bleach Catalysts - The present invention compositions and methods utilize metal-containing bleach catalysts that are effective for use in cleaning compositions. Preferred are manganese and cobalt-containing bleach catalysts.

One type of metal-containing bleach catalyst is a catalyst system comprising a transition metal cation of defined bleach catalytic activity, such as copper, iron, titanium, ruthenium tungsten, molybdenum, or manganese cations, an auxiliary metal cation having little or no bleach catalytic activity, such as zinc or aluminum cations, and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof. Such catalysts are disclosed in U.S. 4,430,243 Bragg, issued February 2, 1982.

Manganese Metal Complexes

If desired, the compositions herein can be catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art and include, for example, the manganese-based catalysts disclosed in U.S. 5,576,282 Miracle et al., issued November 19, 1996; U.S. 5,246,621 Favre et al., issued September 21, 1993; U.S. 5,244,594 Favre et al., issued September 14, 1993; U.S. 5,194,416 Jureller et al., issued March 16, 1993; U.S. 5,114,606 van Vliet et al., issued May 19, 1992; and European Pat. App. Pub. Nos. 549,271 A1, 549,272 A1, 544,440 A2, and 544,490 A1; Preferred examples of these catalysts include $Mn^{IV}_2(u\text{-}O)_3$(1,4,7-trimethyl-1,4,7-triazacyclononane)$_2(PF_6)_2$, $Mn^{III}_2(u\text{-}O)_1(u\text{-}OAc)_2$(1,4,7-trimethyl-1,4,7-triazacyclononane)$_2(ClO_4)_2$, $Mn^{IV}_4(u\text{-}O)_6$(1,4,7-triazacyclononane)$_4(ClO_4)_4$, $Mn^{III}Mn^{IV}_4(u\text{-}O)_1(u\text{-}OAc)_2$-(1,4,7-trimethyl-1,4,7-triazacyclononane)$_2(ClO_4)_3$, Mn^{IV}(1,4,7-trimethyl-1,4,7-triazacyclononane)- $(OCH_3)_3(PF_6)$, and mixtures thereof. Other metal-based

bleach catalysts include those disclosed in U.S. 4,430,243 included by reference herein above and U.S. 5,114,611 van Kralingen, issued May 19, 1992. The use of manganese with various complex ligands to enhance bleaching is also reported in the following: U.S. 4,728,455 Rerek, issued March 1, 1988; U.S. 5,284,944 Madison, issued Februaary 8, 1994; U.S. 5,246,612 van Dijk et al., issued September 21, 1993; U.S. 5,256,779 Kerschner et al., issued October 26, 2993; U.S. 5,280,117 Kerschner et al., issued January 18, 1994; U.S. 5,274,147 Kerschner et al., issued December 28, 1993; U.S. 5,153,161 Kerschner et al., issued October 6, 1992; and U.S. 5,227,084 Martens et al., issued July 13, 1993.

Cobalt Metal Complexes

Cobalt bleach catalysts useful herein are known, and are described, for example, in U.S. 5,597,936 Perkins et al., issued January 28, 1997; U.S. 5,595,967 Miracle et al., January 21, 1997; U.S. 5,703,030 Perkins et al., issued December 30, 1997; and M. L. Tobe, "Base Hydrolysis of Transition-Metal Complexes", Adv. Inorg. Bioinorg. Mech., (1983), 2, pages 1-94. The most preferred cobalt catalyst useful herein are cobalt pentaamine acetate salts having the formula $[Co(NH_3)_5OAc]\ T_y$, wherein "OAc" represents an acetate moiety and "T_y" is an anion, and especially cobalt pentaamine acetate chloride, $[Co(NH_3)_5OAc]Cl_2$; as well as $[Co(NH_3)_5OAc](OAc)_2$; $[Co(NH_3)_5OAc](PF_6)_2$; $[Co(NH_3)_5OAc](SO_4)$; $[Co(NH_3)_5OAc](BF_4)_2$; and $[Co(NH_3)_5OAc](NO_3)_2$ (herein "PAC").

These cobalt catalysts are readily prepared by known procedures, such as taught for example in U.S. 5,597,936, U.S. 5,595,967, U.S. 5,703,030, cited herein above, the Tobe article and the references cited therein, and in U.S. Patent 4,810,410, to Diakun et al, issued March 7,1989, J. Chem. Ed. (1989), 66 (12), 1043-45; The Synthesis and Characterization of Inorganic Compounds, W.L. Jolly (Prentice-Hall; 1970), pp. 461-3; Inorg. Chem., 18, 1497-1502 (1979); Inorg. Chem., 21, 2881-2885 (1982); Inorg. Chem., 18, 2023-2025 (1979); Inorg. Synthesis, 173-176 (1960); and Journal of Physical Chemistry, 56, 22-25 (1952).

Transition Metal Complexes of Macropolycyclic Rigid Ligands - Compositions herein may also suitably include as bleach catalyst a transition metal complex of a macropolycyclic rigid ligand. The phrase "macropolycyclic rigid ligand" is sometimes abbreviated as "MRL" in discussion below. The amount used is a catalytically effective amount, suitably about 1 ppb or more, for example up to about 99.9%, more typically about 0.001 ppm or more, preferably from about 0.05 ppm to about 500 ppm (wherein "ppb" denotes parts per billion by weight and "ppm" denotes parts per million by weight).

Suitable transition metals e.g., Mn are illustrated hereinafter. "Macropolycyclic" means a MRL is both a macrocycle and is polycyclic. "Polycyclic" means at least bicyclic. The term

"rigid" as used herein herein includes "having a superstructure" and "cross-bridged". "Rigid" has been defined as the constrained converse of flexibility: see D.H. Busch., Chemical Reviews., (1993), 93, 847-860, incorporated by reference. More particularly, "rigid" as used herein means that the MRL must be determinably more rigid than a macrocycle ("parent macrocycle") which is otherwise identical (having the same ring size and type and number of atoms in the main ring) but lacking a superstructure (especially linking moieties or, preferably cross-bridging moieties) found in the MRL's. In determining the comparative rigidity of macrocycles with and without superstructures, the practitioner will use the free form (not the metal-bound form) of the macrocycles. Rigidity is well-known to be useful in comparing macrocycles; suitable tools for determining, measuring or comparing rigidity include computational methods (see, for example, Zimmer, Chemical Reviews, (1995), 95(38), 2629-2648 or Hancock et al., Inorganica Chimica Acta, (1989), 164, 73-84.

Preferred MRL's herein are a special type of ultra-rigid ligand which is cross-bridged. A "cross-bridge" is nonlimitingly illustrated in 1.11 hereinbelow. In 1.11, the cross-bridge is a $-CH_2CH_2-$ moiety. It bridges N^1 and N^8 in the illustrative structure. By comparison, a "same-side" bridge, for example if one were to be introduced across N^1 and N^{12} in 1.11, would not be sufficient to constitute a "cross-bridge" and accordingly would not be preferred.

Suitable metals in the rigid ligand complexes include Mn(II), Mn(III), Mn(IV), Mn(V), Fe(II), Fe(III), Fe(IV), Co(I), Co(II), Co(III), Ni(I), Ni(II), Ni(III), Cu(I), Cu(II), Cu(III), Cr(II), Cr(III), Cr(IV), Cr(V), Cr(VI), V(III), V(IV), V(V), Mo(IV), Mo(V), Mo(VI), W(IV), W(V), W(VI), Pd(II), Ru(II), Ru(III), and Ru(IV). Preferred transition-metals in the instant transition-metal bleach catalyst include manganese, iron and chromium.

More generally, the MRL's (and the corresponding transition-metal catalysts) herein suitably comprise:

(a) at least one macrocycle main ring comprising four or more heteroatoms; and

(b) a covalently connected non-metal superstructure capable of increasing the rigidity of the macrocycle, preferably selected from

(i) a bridging superstructure, such as a linking moiety;

(ii) a cross-bridging superstructure, such as a cross-bridging linking moiety; and

(iii) combinations thereof.

The term "superstructure" is used herein as defined in the literature by Busch et al., see, for example, articles by Busch in "Chemical Reviews".

Preferred superstructures herein not only enhance the rigidity of the parent macrocycle, but also favor folding of the macrocycle so that it co-ordinates to a metal in a cleft. Suitable superstructures can be remarkably simple, for example a linking moiety such as any of those illustrated in Fig. 1 and Fig. 2 below, can be used.

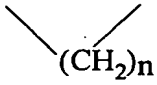


Fig. 1

wherein n is an integer, for example from 2 to 8, preferably less than 6, typically 2 to 4, or

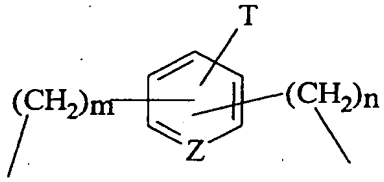


Fig. 2

wherein m and n are integers from about 1 to 8, more preferably from 1 to 3; Z is N or CH; and T is a compatible substituent, for example H, alkyl, trialkylammonium, halogen, nitro, sulfonate, or the like. The aromatic ring in 1.10 can be replaced by a saturated ring, in which the atom in Z connecting into the ring can contain N, O, S or C.

Suitable MRL's are further nonlimitingly illustrated by the following compound:

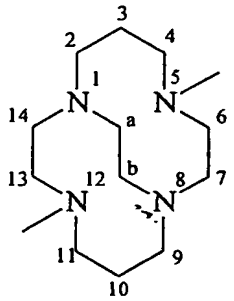


Fig. 3

This is a MRL in accordance with the invention which is a highly preferred, cross-bridged, methyl-substituted (all nitrogen atoms tertiary) derivative of cyclam. Formally, this ligand is named 5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane using the extended von Baeyer system. See "A Guide to IUPAC Nomenclature of Organic Compounds: Recommendations 1993", R. Panico, W.H. Powell and J-C Richer (Eds.), Blackwell Scientific Publications, Boston, 1993; see especially section R-2.4.2.1.

Transition-metal bleach catalysts of Macrocyclic Rigid Ligands which are suitable for use in the invention compositions can in general include known compounds where they conform with the definition herein, as well as, more preferably, any of a large number of novel compounds expressly designed for the present laundry or cleaning uses, and non-limitingly illustrated by any of the following:

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II) Hexafluorophosphate

Aquo-hydroxy-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(III) Hexafluorophosphate

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II) Tetrafluoroborate

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(III) Hexafluorophosphate

Dichloro-5,12-di-n-butyl-1,5,8,12-tetraaza bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5,12-dibenzyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza-bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-octyl-12-methyl-1,5,8,12-tetraaza-bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza-bicyclo[6.6.2]hexadecane Manganese(II).

As a practical matter, and not by way of limitation, the compositions and cleaning processes herein can be adjusted to provide on the order of at least one part per hundred million of the active bleach catalyst species in the aqueous washing medium, and will preferably provide from about 0.01 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the bleach catalyst species in the wash liquor. In order to obtain such levels in the wash liquor of an automatic washing process, typical compositions herein will comprise from about 0.0005% to about 0.2%, more preferably from about 0.004% to about 0.08%, of bleach catalyst, especially manganese or cobalt catalysts, by weight of the cleaning compositions.

ADJUNCT INGREDIENTS

The following are non-limiting examples of adjunct ingredients useful in the laundry compositions of the present invention, said adjunct ingredients include builders, optical brighteners, soil release polymers, dye transfer agents, dispersents, enzymes, suds suppressers, dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, hydrolyzable surfactants, preservatives, anti-oxidants, chelants, stabilizers, anti-

shrinkage agents, anti-wrinkle agents, germicides, fungicides, anti corrosion agents, and mixtures thereof.

Builders - The laundry detergent compositions of the present invention preferably comprise one or more detergent builders or builder systems. When present, the compositions will typically comprise at least about 1% builder, preferably from about 5%, more preferably from about 10% to about 80%, preferably to about 50%, more preferably to about 30% by weight, of detergent builder.

The level of builder can vary widely depending upon the end use of the composition and its desired physical form. When present, the compositions will typically comprise at least about 1% builder. Formulations typically comprise from about 5% to about 50%, more typically about 5% to about 30%, by weight, of detergent builder. Granular formulations typically comprise from about 10% to about 80%, more typically from about 15% to about 50% by weight, of the detergent builder. Lower or higher levels of builder, however, are not meant to be excluded.

Inorganic or P-containing detergent builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates (exemplified by the tripolyphosphates, pyrophosphates, and glassy polymeric meta-phosphates), phosphonates, phytic acid, silicates, carbonates (including bicarbonates and sesquicarbonates), sulphates, and aluminosilicates. However, non-phosphate builders are required in some locales. Importantly, the compositions herein function surprisingly well even in the presence of the so-called "weak" builders (as compared with phosphates) such as citrate, or in the so-called "underbuilt" situation that may occur with zeolite or layered silicate builders.

Examples of silicate builders are the alkali metal silicates, particularly those having a SiO_2:Na_2O ratio in the range 1.6:1 to 3.2:1 and layered silicates, such as the layered sodium silicates described in U.S. 4,664,839 Rieck, issued May 12, 1987. NaSKS-6 is the trademark for a crystalline layered silicate marketed by Hoechst (commonly abbreviated herein as "SKS-6"). Unlike zeolite builders, the Na SKS-6 silicate builder does not contain aluminum. NaSKS-6 has the delta-Na_2SiO_5 morphology form of layered silicate. It can be prepared by methods such as those described in German DE-A-3,417,649 and DE-A-3,742,043. SKS-6 is a highly preferred layered silicate for use herein, but other such layered silicates, such as those having the general formula $NaMSi_xO_{2x+1}\cdot yH_2O$ wherein M is sodium or hydrogen, x is a number from 1.9 to 4, preferably 2, and y is a number from 0 to 20, preferably 0 can be used herein. Various other layered silicates from Hoechst include NaSKS-5, NaSKS-7 and NaSKS-11, as the alpha, beta and gamma forms. As noted above, the delta-Na_2SiO_5 (NaSKS-6 form) is most preferred for

use herein. Other silicates may also be useful such as for example magnesium silicate, which can serve as a crispening agent in granular formulations, as a stabilizing agent for oxygen bleaches, and as a component of suds control systems.

Examples of carbonate builders are the alkaline earth and alkali metal carbonates as disclosed in German Patent Application No. 2,321,001 published on November 15, 1973.

Aluminosilicate builders are useful in the present invention. Aluminosilicate builders are of great importance in most currently marketed heavy duty granular detergent compositions, and can also be a significant builder ingredient in liquid detergent formulations. Aluminosilicate builders include those having the empirical formula:

$$[M_z(zAlO_2)_y]\cdot xH_2O$$

wherein z and y are integers of at least 6, the molar ratio of z to y is in the range from 1.0 to about 0.5, and x is an integer from about 15 to about 264.

Useful aluminosilicate ion exchange materials are commercially available. These aluminosilicates can be crystalline or amorphous in structure and can be naturally-occurring aluminosilicates or synthetically derived. A method for producing aluminosilicate ion exchange materials is disclosed in U.S. 3,985,669, Krummel et al, issued October 12, 1976. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designations Zeolite A, Zeolite P (B), Zeolite MAP and Zeolite X. In an especially preferred embodiment, the crystalline aluminosilicate ion exchange material has the formula:

$$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]\cdot xH_2O$$

wherein x is from about 20 to about 30, especially about 27. This material is known as Zeolite A. Dehydrated zeolites (x = 0 - 10) may also be used herein. Preferably, the aluminosilicate has a particle size of about 0.1-10 microns in diameter.

Organic detergent builders suitable for the purposes of the present invention include, but are not restricted to, a wide variety of polycarboxylate compounds. As used herein, "polycarboxylate" refers to compounds having a plurality of carboxylate groups, preferably at least 3 carboxylates. Polycarboxylate builder can generally be added to the composition in acid form, but can also be added in the form of a neutralized salt. When utilized in salt form, alkali metals, such as sodium, potassium, and lithium, or alkanolammonium salts are preferred.

Included among the polycarboxylate builders are a variety of categories of useful materials. One important category of polycarboxylate builders encompasses the ether polycarboxylates, including oxydisuccinate, as disclosed in U.S. 3,128,287 Berg, issued April 7, 1964, U.S. 3,635,830 Lamberti et al., issued January 18, 1972, and U.S. 3,936,448 Lamberti, issued February 3, 1976. See also "TMS/TDS" builders of U.S. 4,663,071 Bush et al., issued May 5,

1987. Suitable ether polycarboxylates also include cyclic compounds, particularly alicyclic compounds, such as those described in U.S. 3,923,679 Rapko, issued December 2, 1975; U.S. 4,158,635 Crutchfield et al., issued June 19, 1979; U.S. 4,120,874 Crutchfield et al., issued October 17, 1978; and U.S. 4,102,903 Crutchfield et al., issued July 25, 1978.

Other useful detergency builders include the ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof.

Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders of particular importance for heavy duty liquid detergent formulations due to their availability from renewable resources and their biodegradability. Citrates can also be used in granular compositions, especially in combination with zeolite and/or layered silicate builders. Oxydisuccinates are also especially useful in such compositions and combinations.

Also suitable in the detergent compositions of the present invention are the 3,3-dicarboxy-4-oxa-1,6-hexanedioates and the related compounds disclosed in U.S. 4,566,984, Bush, issued January 28, 1986. Useful succinic acid builders include the C_5-C_{20} alkyl and alkenyl succinic acids and salts thereof. A particularly preferred compound of this type is dodecenylsuccinic acid. Specific examples of succinate builders include: laurylsuccinate, myristylsuccinate, palmitylsuccinate, 2-dodecenylsuccinate (preferred), 2-pentadecenylsuccinate, and the like. Laurylsuccinates are the preferred builders of this group, and are described in European Patent Application 86200690.5/0,200,263, published November 5, 1986.

Other suitable polycarboxylates are disclosed in U.S. 4,144,226, Crutchfield et al., issued March 13, 1979 and in U.S. 3,308,067, Diehl, issued March 7, 1967. See also Diehl U.S. Patent 3,723,322.

Fatty acids, e.g., C_{12}-C_{18} monocarboxylic acids, can also be incorporated into the compositions alone, or in combination with the aforesaid builders, especially citrate and/or the succinate builders, to provide additional builder activity. Such use of fatty acids will generally result in a diminution of sudsing, which should be taken into account by the formulator.

In situations where phosphorus-based builders can be used, and especially in the formulation of bars used for hand-laundering operations, the various alkali metal phosphates such as the well-known sodium tripolyphosphates, sodium pyrophosphate and sodium orthophosphate

can be used. Phosphonate builders such as ethane-1-hydroxy-1,1-diphosphonate and other known phosphonates (see, for example, U.S. Patents 3,159,581; 3,213,030; 3,422,021; 3,400,148 and 3,422,137) can also be used.

Chelating Agents

The detergent compositions herein may also optionally contain one or more iron and/or manganese chelating agents. Such chelating agents can be selected from the group consisting of amino carboxylates, amino phosphonates, polyfunctionally-substituted aromatic chelating agents and mixtures therein, all as hereinafter defined. Without intending to be bound by theory, it is believed that the benefit of these materials is due in part to their exceptional ability to remove iron and manganese ions from washing solutions by formation of soluble chelates.

Examples of suitable chelating agents and levels of use are described in U.S. Pat. Nos. 5,576,282 and 5,728,671.

A preferred biodegradable chelator for use herein is ethylenediamine disuccinate ("EDDS"), especially the [S,S] isomer as described in U.S. Patent 4,704,233, November 3, 1987, to Hartman and Perkins.

The compositions herein may also contain water-soluble methyl glycine diacetic acid (MGDA) salts (or acid form) as a chelant or co-builder useful with, for example, insoluble builders such as zeolites, layered silicates and the like.

If utilized, these chelating agents will generally comprise from about 0.1% by weight of the detergent compositions herein to about 15%, more preferably 3.0% by weight of the detergent compositions herein.

Dye Transfer Inhibiting Agents

The detergent compositions of the present invention may also include one or more compounds, dye transfer inhibiting agents, for inhibiting dye transfer from one fabric to another of solubilized and suspended dyes encountered during fabric laundering and conditioning operations involving colored fabrics.

Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. Examples of such dye transfer inhibiting agents are disclosed in U.S. Pat. Nos. 5,707,950 and 5,707,951.

Additional suitable dye transfer inhibiting agents include, but are not limited to, cross-linked polymers. Cross-linked polymers are polymers whose backbone are interconnected to a certain degree; these links can be of chemical or physical nature, possibly with active groups on

the backbone or on branches. Cross-linked polymers have been described in the Journal of Polymer Science, volume 22, pages 1035-1039.

In one embodiment, the cross-linked polymers are made in such a way that they form a three-dimensional rigid structure, which can entrap dyes in the pores formed by the three-dimensional structure.

In another embodiment, the cross-linked polymers entrap dyes by swelling.

Suitable cross-linked polymers are described in the co-pending European patent application 94870213.9.

Addition of such polymers also enhances the performance of the enzymes within the detergent compositions herein.

The dye transfer inhibiting agents have the ability to complex or adsorb fugitive dyes wash out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

When present in the detergent compositions herein, the dye transfer inhibiting agents are present at levels from about 0.0001%, more preferably about 0.01%, most preferably about 0.05% by weight of the detergent compositions to about 10%, more preferably about 2%, most preferably about 1% by weight of the detergent compositions.

Dispersants

The detergent composition of the present invention can also contain dispersants. Suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 1,000 to 100,000.

Especially, copolymer of acrylate and methylacrylate such as the 480N having a molecular weight of 4000, at a level from 0.5-20% by weight of composition can be added in the detergentcompositions of the present invention.

The compositions of the invention may contain a lime soap peptiser compound, which has a lime soap dispersing power (LSDP), as defined hereinafter of no more than 8, preferably no more than 7, most preferably no more than 6. The lime soap peptiser compound is preferably present at a level from 0% to 20% by weight.

A numerical measure of the effectiveness of a lime soap peptiser is given by the lime soap dispersant power (LSDP) which is determined using the lime soap dispersant test as

described in an article by H.C. Borghetty and C.A. Bergman, J. Am. Oil. Chem. Soc., volume 27, pages 88-90, (1950). This lime soap dispersion test method is widely used by practitioners in this art field being referred to, for example, in the following review articles; W.N. Linfield, Surfactant science Series, Volume 7, page 3; W.N. Linfield, Tenside surf. det., volume 27, pages 159-163, (1990); and M.K. Nagarajan, W.F. Masler, Cosmetics and Toiletries, volume 104, pages 71-73, (1989). The LSDP is the % weight ratio of dispersing agent to sodium oleate required to disperse the lime soap deposits formed by 0.025g of sodium oleate in 30ml of water of 333ppm $CaCo_3$ (Ca:Mg=3:2) equivalent hardness.

Surfactants having good lime soap peptiser capability will include certain amine oxides, betaines, sulfobetaines, alkyl ethoxysulfates and ethoxylated alcohols.

Exemplary surfactants having a LSDP of no more than 8 for use in accord with the present invention include C_{16}-C_{18} dimethyl amine oxide, C_{12}-C_{18} alkyl ethoxysulfates with an average degree of ethoxylation of from 1-5, particularly C_{12}-C_{15} alkyl ethoxysulfate surfactant with a degree of ethoxylation of amount 3 (LSDP=4), and the C_{14}-C_{15} ethoxylated alcohols with an average degree of ethoxylation of either 12 (LSDP=6) or 30, sold under the tradenames Lutensol A012 and Lutensol A030 respectively, by BASF GmbH.

Polymeric lime soap peptisers suitable for use herein are described in the article by M.K. Nagarajan, W.F. Masler, to be found in Cosmetics and Toiletries, volume 104, pages 71-73, (1989).

Hydrophobic bleaches such as 4-[N-octanoyl-6-aminohexanoyl]benzene sulfonate, 4-[N-nonanoyl-6-aminohexanoyl]benzene sulfonate, 4-[N-decanoyl-6-aminohexanoyl]benzene sulfonate and mixtures thereof; and nonanoyloxy benzene sulfonate together with hydrophilic / hydrophobic bleach formulations can also be used as lime soap peptisers compounds.

Conventional detergent enzymes

The detergent compositions can comprise in addition to the amylase of the present invention one or more detergent enzymes which provide cleaning performance and/or fabric care benefits. Such enzymes can include proteases, amylases, cellulases and lipases. Such materials are known in the art and are commercially available under such trademarks as . They may be incorporated into the non-aqueous liquid detergent compositions herein in the form of suspensions, "marumes" or "prills". Another suitable type of enzyme comprises those in the form of slurries of enzymes in nonionic surfactants, e.g., the enzymes marketed by Novo Nordisk under the tradename "SL" or the microencapsulated enzymes marketed by Novo Nordisk under

the tradename "LDP." Suitable enzymes and levels of use are described in U.S. Pat. No. 5,576,282.

Enzymes added to the compositions herein in the form of conventional enzyme prills are especially preferred for use herein. Such prills will generally range in size from about 100 to 1,000 microns, more preferably from about 200 to 800 microns and will be suspended throughout the non-aqueous liquid phase of the composition. Prills in the compositions of the present invention have been found, in comparison with other enzyme forms, to exhibit especially desirable enzyme stability in terms of retention of enzymatic activity over time. Thus, compositions which utilize enzyme prills need not contain conventional enzyme stabilizing such as must frequently be used when enzymes are incorporated into aqueous liquid detergents.

Examples of suitable enzymes include, but are not limited to, hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, keratanases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and known amylases, or mixtures thereof. A preferred combination is a detergent composition having a cocktail of conventional applicable enzymes like protease, lipase, cutinase and/or cellulase in conjunction with the amylase of the present invention.

Examples of such suitable enzymes are disclosed in U.S. Patent Nos. 5,576,282, 5,728,671 and 5,707,950

Suitable proteases are the subtilisins which are obtained from particular strains of *B. subtilis* and *B. licheniformis* (subtilisin BPN and BPN'). One suitable protease is obtained from a strain of *Bacillus*, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE® by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Other suitable proteases include ALCALASE®, DURAZYM® and SAVINASE® from Novo and MAXATASE®, MAXACAL®, PROPERASE® and MAXAPEM® (protein engineered Maxacal) from Gist-Brocades. Proteolytic enzymes also encompass modified bacterial serine proteases, such as those described in European Patent Application Serial Number 87 303761.8, filed April 28, 1987 (particularly pages 17, 24 and 98), and which is called herein "Protease B", and in European Patent Application 199,404, Venegas, published October 29, 1986, which refers to a modified bacterial serine protealytic enzyme which is called "Protease A" herein. More preferred is what is called herein "Protease C", which is a variant of an alkaline serine protease from *Bacillus* in which lysine replaced arginine at position 27, tyrosine replaced valine at position 104, serine replaced asparagine at position 123, and alanine replaced threonine at

position 274. Protease C is described in EP 90915958:4, corresponding to WO 91/06637, Published May 16, 1991. Genetically modified variants, particularly of Protease C, are also included herein. See also a high pH protease from Bacillus sp. NCIMB 40338 described in WO 93/18140 A to Novo. Enzymatic detergents comprising protease, one or more other enzymes, and a reversible protease inhibitor are described in WO 92/03529 A to Novo. When desired, a protease having decreased adsorption and increased hydrolysis is available as described in WO 95/07791 to Procter & Gamble. A recombinant trypsin-like protease for detergents suitable herein is described in WO 94/25583 to Novo.

In more detail, the protease referred to as "Protease D" is a carbonyl hydrolase variant having an amino acid sequence not found in nature, which is derived from a precursor carbonyl hydrolase by substituting a different amino acid for a plurality of amino acid residues at a position in said carbonyl hydrolase equivalent to position +76, preferably also in combination with one or more amino acid residue positions equivalent to those selected from the group consisting of +99, +101, +103, +104, +107, +123, +27, +105, +109, +126, +128, +135, +156, +166, +195, +197, +204, +206, +210, +216, +217, +218, +222, +260, +265, and/or +274 according to the numbering of *Bacillus amyloliquefaciens* subtilisin, as described in WO 95/10615 published April 20, 1995 by Genencor International. Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO91/06637 and protease BLAP® described in WO91/02792. The proteolytic enzymes are incorporated in the detergent compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.001% to 0.2%, more preferably from 0.005% to 0.1% pure enzyme by weight of the composition.

Useful proteases are also described in PCT publications: WO 95/30010 published November 9, 1995 by The Procter & Gamble Company; WO 95/30011 published November 9, 1995 by The Procter & Gamble Company; WO 95/29979 published November 9, 1995 by The Procter & Gamble Company.

The cellulases usable in the present invention include both bacterial or fungal cellulase. Preferably, they will have a pH optimum of between 5 and 9.5. Suitable cellulases are disclosed in U.S. Patent 4,435,307, Barbesgoard et al, which discloses fungal cellulase produced from Humicola insolens. Suitable cellulases are also disclosed in GB-A-2.075.028; GB-A-2.095.275 and DE-OS-2.247.832.

Examples of such cellulases are cellulases produced by a strain of Humicola insolens (Humicola grisea var. thermoidea), particularly the Humicola strain DSM 1800.

Other suitable cellulases are cellulases originated from Humicola insolens having a molecular weight of about 50KDa, an isoelectric point of 5.5 and containing 415 amino acids; and a ~43kD endoglucanase derived from Humicola insolens, DSM 1800, exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO 91/17243. Also suitable cellulases are the EGIII cellulases from Trichoderma longibrachiatum described in WO94/21801, Genencor, published September 29, 1994. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are cellulases described in European patent application No. 91202879.2, filed November 6, 1991 (Novo). Carezyme and Celluzyme (Novo Nordisk A/S) are especially useful. See also WO91/17243.

Peroxidase enzymes are known in the art, and include, for example, horseradish peroxidase, ligninase and haloperoxidase such as chloro- and bromo-peroxidase. Peroxidase-containing detergent compositions are disclosed, for example, in U.S. Patent Nos. 5,576,282, 5,728,671 and 5,707,950, PCT International Applications WO 89/099813, WO89/09813 and in European Patent application EP No. 91202882.6, filed on November 6, 1991 and EP No. 96870013.8, filed February 20, 1996. Also suitable is the laccase enzyme.

Preferred enhancers are substitued phenthiazine and phenoxasine 10-Phenothiazinepropionicacid (PPT), 10-ethylphenothiazine-4-carboxylic acid (EPC), 10-phenoxazinepropionic acid (POP) and 10-methylphenoxazine (described in WO 94/12621) and substitued syringates (C3-C5 substitued alkyl syringates) and phenols. Sodium percarbonate or perborate are preferred sources of hydrogen peroxide.

Said peroxidases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Other preferred enzymes that can be included in the detergent compositions of the present invention include lipases. Suitable lipase enzymes for detergent usage include those produced by microorganisms of the Pseudomonas group, such as Pseudomonas stutzeri ATCC 19.154, as disclosed in British Patent 1,372,034. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism *Pseudomonas fluorescent* IAM 1057. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P". Other suitable commercial lipases include Amano-CES, lipases ex *Chromobacter viscosum*, e.g. *Chromobacter viscosum var. lipolyticum* NRRLB 3673 from Toyo Jozo Co., Tagata, Japan; *Chromobacter viscosum* lipases from U.S. Biochemical Corp., U.S.A.

and Disoynth Co., The Netherlands, and lipases ex *Pseudomonas gladioli*. Especially suitable lipases are lipases such as M1 LIPASE® and LIPOMAX® (Gist-Brocades) and LIPOLASE® and LIPOLASE ULTRA®(Novo) which have found to be very effective when used in combination with the compositions of the present invention.

Also suitable are cutinases [EC 3.1.1.50] which can be considered as a special kind of lipase, namely lipases which do not require interfacial activation. Addition of cutinases to detergent compositions have been described in e.g. WO 88/09367 (Genencor).

The lipases and/or cutinases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Known amylases (α and/or ß) can be included for removal of carbohydrate-based stains. WO 94/02597, Novo Nordisk A/S published February 03, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO94/18314, Genencor, published August 18, 1994 and WO95/10603, Novo Nordisk A/S, published April 20, 1995. Other amylases known for use in detergent compositions include both α- and β-amylases. α-Amylases are known in the art and include those disclosed in US Pat. 5,003,257; EP 252,666; WO 91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent Specification No. 1,296,839 (Novo). Other suitable amylase are stability-enhanced amylases including PURAFACT OX AM® described in WO 94/18314, published August 18, 1994 and WO96/05295, Genencor, published Februaury 22, 1996 and amylase variants from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95.

Examples of commercial α-amylases products are TERMAMYL®, BAN®, FUNGAMYL® and DURAMYL®, all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases : α-amylases characterized by having a specific activity at least 25% higher than the specific activity of TERMAMYL® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas® α-amylase activity assay. Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Purified or non-purified forms of these enzymes may be used. Also included by definition, are mutants of native enzymes. Mutants can be obtained e.g. by protein and/or genetic engineering, chemical and/or physical modifications of native enzymes.

Common practice as well is the expression of the enzyme via host organisms in which the genetic material responsible for the production of the enzyme has been cloned.

Said enzymes are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition. The enzymes can be added as separate single ingredients (prills, granulates, stabilized liquids, etc. containing one enzyme) or as mixtures of two or more enzymes (e.g. cogranulates).

Other suitable detergent ingredients that can be added are enzyme oxidation scavengers. Examples of such enzyme oxidation scavengers are ethoxylated tetraethylene polyamines.

A range of enzyme materials and means for their incorporation into synthetic detergent compositions is also disclosed in WO 93/07263 and WO 93/07260 to Genencor International, WO 89/08694 to Novo, and U.S. 3,553,139, January 5, 1971 to McCarty et al. Enzymes are further disclosed in U.S. 4,101,457, Place et al, July 18, 1978, and in U.S. 4,507,219, Hughes, March 26, 1985. Enzyme materials useful for liquid detergent formulations, and their incorporation into such formulations, are disclosed in U.S. 4,261,868, Hora et al, April 14, 1981.

Enzyme Stabilizers

Enzymes for use in detergents can be stabilized by various techniques. Enzyme stabilization techniques are disclosed and exemplified in U.S. 3,600,319, August 17, 1971, Gedge et al, EP 199,405 and EP 200,586, October 29, 1986, Venegas. Enzyme stabilization systems are also described, for example, in U.S. 3,519,570. A useful Bacillus, sp. AC13 giving proteases, xylanases and cellulases, is described in WO 9401532 to Novo. The enzymes employed herein can be stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions which provide such ions to the enzymes. Suitable enzyme stabilizers and levels of use are described in U.S. Pat. No. 5,576,282.

Other Detergent Ingredients

The detergent compositions herein may also optionally contain one or more of the following: polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids and/or pigments. Suitable examples of such other detergent ingredients and levels of use are found in U.S. Patent No. 5,576,282.

Method of washing

The compositions of the invention may be used in essentially any washing or cleaning methods, including soaking methods, pretreatment methods and methods with rinsing steps for which a separate rinse aid composition may be added.

The process described herein comprises contacting fabrics with a laundering solution in the usual manner and exemplified hereunder.

The process of the invention is conveniently carried out in the course of the cleaning process. The method of cleaning is preferably carried out at 5°C to 95°C, especially between 10°C and 60°C. The pH of the treatment solution is preferably from 7 to 11.

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention. In the detergent compositions, the enzyme levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications herein have the following meanings:

LAS	:	Sodium linear C_{12} alkyl benzene sulphonate
TAS	:	Sodium tallow alkyl sulphate
CXYAS	:	Sodium C_{1X} - C_{1Y} alkyl sulfate
25EY	:	A C_{12}-C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide
CXYEZ	:	A C_{1X} - C_{1Y} predominantly linear primary alcohol condensed with an average of Z moles of ethylene oxide
XYEZS	:	C_{1X} - C_{1Y} sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole
QAS	:	$R_2.N^+(CH_3)_2(C_2H_4OH)$ with $R_2 = C_{12}$-C_{14}
Soap	:	Sodium linear alkyl carboxylate derived from a 80/20 mixture of tallow and coconut oils.

Nonionic	:	C_{13}-C_{15} mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5 sold under the tradename Plurafac LF404 by BASF Gmbh.
CFAA	:	C_{12}-C_{14} alkyl N-methyl glucamide
TFAA	:	C_{16}-C_{18} alkyl N-methyl glucamide.
TPKFA	:	C12-C14 topped whole cut fatty acids.
DEQA	:	Di-(tallow-oxy-ethyl) dimethyl ammonium chloride.
Neodol 45-13	:	C14-C15 linear primary alcohol ethoxylate, sold by Shell Chemical CO.
Silicate	:	Amorphous Sodium Silicate (SiO_2:Na_2O ratio = 2.0)
NaSKS-6	:	Crystalline layered silicate of formula δ-$Na_2Si_2O_5$.
Carbonate	:	Anhydrous sodium carbonate with a particle size between 200 μm and 900μm.
Bicarbonate	:	Anhydrous sodium bicarbonate with a particle size between 400 μm and 1200μm.
STPP	:	Anhydrous sodium tripolyphosphate
MA/AA	:	Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 70,000-80,000

Zeolite A	:	Hydrated Sodium Aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}$. $27H_2O$ having a primary particle size in the range from 0.1 to 10 micrometers
Citrate	:	Tri-sodium citrate dihydrate of activity 86,4% with a particle size distribution between 425 μm and 850 μm.
Citric	:	Anhydrous citric acid
PB1	:	Anhydrous sodium perborate monohydrate bleach, empirical formula $NaBO_2.H_2O_2$
PB4	:	Anhydrous sodium perborate tetrahydrate
Percarbonate	:	Anhydrous sodium percarbonate bleach of empirical formula $2Na_2CO_3.3H_2O_2$
TAED	:	Tetraacetyl ethylene diamine.
NOBS	:	Nonanoyloxybenzene sulfonate in the form of the sodium salt.
Photoactivated Bleach	:	Sulfonated zinc phtalocyanine encapsulated in dextrin soluble polymer.
Protease	:	Proteolytic enzyme sold under the tradename Savinase, Alcalase, Durazym by Novo Nordisk A/S, Maxacal, Maxapem sold by Gist-Brocades and proteases described in patents WO91/06637 and/or WO95/10591 and/or EP 251,446.
Amylase	:	Amylolytic enzyme prepared by the present invention method having hydrolytic activity 50% faster than that of the amylase enzyme having SEQ. ID. 1.

Lipase	:	Lipolytic enzyme sold under the tradename Lipolase, Lipolase Ultra by Novo Nordisk A/S
Cellulase	:	Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novo Nordisk A/S.
CMC	:	Sodium carboxymethyl cellulose.
HEDP	:	1,1-hydroxyethane diphosphonic acid.
DETPMP	:	Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Trade name Dequest 2060.
PVNO	:	Poly(4-vinylpyridine)-N-Oxide.
PVPVI	:	Poly (4-vinylpyridine)-N-oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone.
Brightener 1	:	Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
Brightener 2	:	Disodium 4,4'-bis(4-anilino-6-morpholino-1.3.5-triazin-2-yl) stilbene-2:2'-disulfonate.
Silicone antifoam	:	Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
Granular Suds Suppressor	:	12% Silicone/silica, 18% stearyl alcohol,70% starch in granular form
SRP 1	:	Sulfobenzoyl or sodium isethionate end capped esters with oxyethylene oxy and terephtaloyl backbone.

SRP 2 : Diethoxylated poly (1,2 propylene terephtalate) short block polymer.

Sulphate : Anhydrous sodium sulphate.

HMWPEO : High molecular weight polyethylene oxide

Example 1

The following detergent formulations, according to the present invention are prepared, where I and III are phosphorus-containing detergent compositions, and II is a zeolite-containing detergent composition:

	I	II	III
Blown Powder:			
STPP	24.0	-	24.0
Zeolite A	-	24.0	-
C45AS	9.0	6.0	13.0
MA/AA	2.0	4.0	2.0
LAS	6.0	8.0	11.0
TAS	2.0	-	-
Silicate	7.0	3.0	3.0
CMC	1.0	1.0	0.5
Brightener 2	0.2	0.2	0.2
Soap	1.0	1.0	1.0
DETPMP	0.4	0.4	0.2
Spray On			
C45E7	2.5	2.5	2.0
C25E3	2.5	2.5	2.0
Silicone antifoam	0.3	0.3	0.3
Perfume	0.3	0.3	0.3
Dry additives:			
Carbonate	6.0	13.0	15.0
PB4	18.0	18.0	10.0

PB1	4.0	4.0	0
TAED	3.0	3.0	1.0
Photoactivated bleach	0.02	0.02	0.02
Protease	0.01	0.01	0.01
Lipase	0.009	0.009	0.009
Amylase	0.002	0.003	0.01
Dry mixed sodium sulfate	3.0	3.0	5.0
Balance (Moisture & Miscellaneous)	100.0	100.0	100.0
Density (g/litre)	630	670	670

Example 2

The following nil bleach-containing detergent formulations of particular use in the washing of colored clothing, according to the present invention are prepared:

	I	II	III
Blown Powder			
Zeolite A	15.0	15.0	-
Sodium sulfate	0.0	5.0	-
LAS	3.0	3.0	-
DETPMP	0.4	0.5	-
CMC	0.4	0.4	-
MA/AA	4.0	4.0	-
Agglomerates			
C45AS	-	-	11.0
LAS	6.0	5.0	-
TAS	3.0	2.0	-
Silicate	4.0	4.0	-
Zeolite A	10.0	15.0	13.0
CMC	-	-	0.5
MA/AA	-	-	2.0
Carbonate	9.0	7.0	7.0
Spray On			
Perfume	0.3	0.3	0.5

C45E7	4.0	4.0	4.0
C25E3	2.0	2.0	2.0
Dry additives			
MA/AA	-	-	3.0
NaSKS-6	-	-	12.0
Citrate	10.0	-	8.0
Bicarbonate	7.0	3.0	5.0
Carbonate	8.0	5.0	7.0
PVPVI/PVNO	0.5	0.5	0.5
Protease	0.026	0.016	0.047
Lipase	0.009	--	0.009
Amylase	0.005	0.05	0.005
Cellulase	0.006	0.006	--
Silicone antifoam	5.0	5.0	5.0
Dry additives			
Sodium sulfate	0.0	9.0	0.0
Balance (Moisture and Miscellaneous)	100.0	100.0	100.0
Density (g/litre)	700	700	700

Example 3

The following detergent formulations, according to the present invention are prepared:

	I	II	III	IV
LAS	20.0	14.0	24.0	22.0
QAS	0.7	1.0	-	0.7
TFAA	-	1.0	-	-
C25E5/C45E7	-	2.0	-	0.5
C45E3S	-	2.5	-	-
STPP	30.0	18.0	30.0	22.0
Silicate	9.0	5.0	10.0	8.0
Carbonate	13.0	7.5	-	5.0
Bicarbonate	-	7.5	-	-
DETPMP	0.7	1.0	-	-

SRP 1	0.3	0.2	-	0.1
MA/AA	2.0	1.5	2.0	1.0
CMC	0.8	0.4	0.4	0.2
Protease	0.008	0.01	0.026	0.026
Amylase	0.007	0.004	0.005	0.002
Lipase	0.004	--	--	0.002
Cellulase	0.0015	0.0005	-	-
Photoactivated bleach	70ppm	45ppm	-	10ppm
Brightener 1	0.2	0.2	0.08	0.2
PB1	6.0	2.0	-	-
NOBS	2.0	1.0	-	-
Balance (Moisture and Miscellaneous)	100	100	100	100

Example 4

The following liquid detergent formulations, according to the present invention are prepared:

	I	II	III	IV	V	VI	VII	VIII
LAS	10.0	13.0	9.0	-	25.0	-	-	-
C25AS	4.0	1.0	2.0	10.0	-	13.0	18.0	15.0
C25E3S	1.0	-	-	3.0	-	2.0	2.0	4.0
C25E7	6.0	8.0	13.0	2.5	-	-	4.0	4.0
TFAA	-	-	-	4.5	-	6.0	8.0	8.0
QAS	-	-	-	-	3.0	1.0	-	-
TPKFA	2.0	-	13.0	2.0	-	15.0	7.0	7.0
Rapeseed fatty acids	-	-	-	5.0	-	-	4.0	4.0
Citric	2.0	3.0	1.0	1.5	1.0	1.0	1.0	1.0
Dodecenyl/ tetradecenyl succinic acid	12.0	10.0	-	-	15.0	-	-	-
Oleic acid	4.0	2.0	1.0	-	1.0	-	-	-
Ethanol	4.0	4.0	7.0	2.0	7.0	2.0	3.0	2.0
1,2 Propanediol	4.0	4.0	2.0	7.0	6.0	8.0	10.0	13.-

Mono Ethanol Amine	-	-	-	5.0	-	-	9.0	9.0
Tri Ethanol Amine	-	-	8	-	-	-	-	-
NaOH (pH)	8.0	8.0	7.6	7.7	8.0	7.5	8.0	8.2
Ethoxylated tetraethylene pentamine	0.5	-	0.5	0.2	-	-	0.4	0.3
DETPMP	1.0	1.0	0.5	1.0	2.0	1.2	1.0	-
SRP 2	0.3	-	0.3	0.1	-	-	0.2	0.1
PVNO	-	-	-	-	-	-	-	0.10
Protease	.005	.005	.004	.003	0.08	.005	.003	.006
Lipase	-	.002	-	.0002	-	-	.003	.003
Amylase	.002	.002	.005	.004	.002	.008	.005	.005
Cellulase	-	-	-	.0001	-	-	.0004	.0004
Boric acid	0.1	0.2	-	2.0	1.0	1.5	2.5	2.5
Na formate	-	-	1.0	-	-	-	-	-
Ca chloride	-	0.015	-	0.01	-	-	-	-
Bentonite clay	-	-	-	-	4.0	4.0	-	-
Suspending clay SD3	-	-	-	-	0.6	0.3	-	-
Balance Moisture and Miscellaneous	100	100	100	100	100	100	100	100

Example 5

Granular fabric detergent compositions which provide "softening through the wash" capability are prepared in accord with the present invention :

	I	II
45AS	-	10.0
LAS	7.6	-
68AS	1.3	-
45E7	4.0	-
25E3	-	5.0

Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride	1.4	1.0
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4
PB1	15.0	-
Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	5.0
HMWPEO	-	0.1
Protease	0.02	0.01
Lipase	0.02	0.01
Amylase	0.01	0.005
Cellulase	0.001	-
Silicate	3.0	5.0
Carbonate	10.0	10.0
Granular suds suppressor	1.0	4.0
CMC	0.2	0.1
Water/minors	Up to 100%	

Example 6

Syndet bar fabric detergent compositions are prepared in accord with the present invention :

	I	II	III	IV
C26 AS	20.00	20.00	20.00	20.00
CFAA	5.0	5.0	5.0	5.0
LAS (C11-13)	10.0	10.0	10.0	10.0
Sodium carbonate	25.0	25.0	25.0	25.0
Sodium pyrophosphate	7.0	7.0	7.0	7.0
STPP	7.0	7.0	7.0	7.0
Zeolite A	5.0	5.0	5.0	5.0
CMC	0.2	0.2	0.2	0.2

Polyacrylate (MW 1400)	0.2	0.2	0.2	0.2
Coconut monethanolamide	5.0	5.0	5.0	5.0
Amylase	0.01	0.02	0.005	0.01
Protease	0.3	-	0.5	0.05
Brightener, perfume	0.2	0.2	0.2	0.2
CaSO4	1.0	1.0	1.0	1.0
MgSO4	1.0	1.0	1.0	1.0
Water	4.0	4.0	4.0	4.0

Filler* : balance to 100%

*Can be selected from convenient materials such as CaCO3, talc, clay (Kaolinite, Smectite), silicates, and the like.

In addition to the above examples, the amylase of the present invention can be formulated into any suitable laundry detergent composition, non-limiting examples of which are described in U.S. 5,679,630 Baeck et al., issued October 21, 1997; U.S. 5,565,145 Watson et al., issued October 15, 1996; U.S. 5,478,489 Fredj et al., issued December 26, 1995; U.S. 5,470,507 Fredj et al., issued November 28, 1995; U.S. 5,466,802 Panandiker et al., issued November 14, 1995; U.S. 5,460,752 Fredj et al., issued October 24, 1995; U.S. 5,458,810 Fredj et al., issued October 17, 1995; U.S. 5,458,809 Fredj et al., issued October 17, 1995; U.S. 5,288,431 Huber et al., issued February 22, 1994 all of which are incorporated herein by reference.

The compositions of the present invention can be suitably prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. 5,691,297 Nassano et al., issued November 11, 1997; U.S. 5,574,005 Welch et al., issued November 12, 1996; U.S. 5,569,645 Dinniwell et al., issued October 29, 1996; U.S. 5,565,422 Del Greco et al., issued October 15, 1996; U.S. 5,516,448 Capeci et al., issued May 14, 1996; U.S. 5,489,392 Capeci et al., issued February 6, 1996; U.S. 5,486,303 Capeci et al., issued January 23, 1996 all of which are incorporated herein by reference.

What is claimed is:

1. A method for producing DNA for new amylase enzymes useful for cleaning applications, said method comprising:

(a) randomly mutating, by chemical and/or enzymatic mean, single or double site mutations in one or more DNA encoding for an amylase enzyme;

(b) incorporating the mutated DNA from step (a) into a microorganism which is capable of expressing amylase enzyme;

(c) separating into individual isolates organisms from step (b) such that a minimum number, preferably on average about one, of the organisms containing mutated DNA is present in each isolate;

(d) producing amylase enzyme from one or more of the isolates;

(e) collecting the mutated DNA from one or more isolates that produce amylase which hydrolyzes starch at a rate of at least about 25% faster than the parent amylase in the presence of cleaning composition ingredients comprising at least surfactants, builders and/or bleaches, preferably at a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1;

(f) optionally, DNA shuffling during a separate step or concurrently with any of steps (a) to (e); and

(g) optionally repeating, one or more times, steps (a) to (f) and then collecting the mutated DNA.

2. A method for producing organisms which produce amylase enzymes useful for cleaning applications, said method comprising:

(a) randomly mutating, by chemical and/or enzymatic mean, single or double site mutations in one or more DNA encoding for an amylase enzyme;

(b) incorporating the mutated DNA from step (a) into a microorganism which is capable of expressing amylase enzyme;

(c) separating into individual isolates organisms from step (b) such that a minimum number, preferably on average about one, of the organisms containing mutated DNA is present in each isolate;

(d) producing amylase enzyme from one or more of the isolates;

(e) collecting the organisms containing mutated DNA from one or more isolates that produce amylase which hydrolyzes starch at a rate of at least about 25% faster than the parent amylase in the presence of cleaning composition ingredients comprising at least surfactants, builders and/or bleaches, preferably at a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1;

(f) optionally, DNA shuffling during a separate step or concurrently with any of steps (a) to (e); and

(g) optionally repeating, one or more times, steps (a) to (f) and then collecting the organisms containing mutated DNA that produce the amylase.

3. Detergent compositions comprising:

(a) from about 0.0001% to about 2% by weight of an amylase enzyme, having a level of hydrolytic activity of at least about 25% faster than that of the amylase enzyme having SEQ. ID. 1, produced by an organism containing mutated DNA prepared by a method according to either of Claims 1 or 2; and

(b) from about 98% to about 99.9999% of cleaning composition ingredients comprising one or more of surfactants, builders, and bleaching agents.

4. The detergent composition according to Claim 3 comprising at least about 5% by weight of anionic surfactant, preferably seleted from alkyl sulfate, alkyl ethoxy sulfate and/or linear alkylene sulfonate.

5. The detergent composition according to either of Claims 3 or 4 comprising at least about 2% of an alkyl ethoxylate nonionic surfactant.

6. The detergent composition according to any of Claims 3-5 comprising cationic and anionic surfactants.

7. The detergent composition according to any of Claims 3-6 comprising a proteolytic and/or cellulolytic enzyme

8. The detergent composition according to any of Claims 3-7 comprising a bleaching agent, preferably selected from the group consisting of percarbonates, perborates and mixtures thereof

and/or bleach activators, more preferably hydrophobic bleach activators selected from the group consisting of tetraacetyl ethylene diamine (TAED), benzoylcaprolactam (BzCL), 4-nitrobenzoylcaprolactam, 3-chlorobenzoylcaprolactam, benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), phenyl benzoate (PhBz), decanoyloxybenzenesulphonate (C_{10}-OBS), benzoylvalerolactam (BZVL), octanoyloxybenzenesulphonate (C_8-OBS), perhydrolyzable esters, 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS), lauryloxybenzenesulphonate (LOBS or C_{12}-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C_{11}-OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA) and mixtures thereof.

9. The detergent composition according to any of Claims 3-8 comprising a dye transfer inhibiting agent.

10. The detergent composition according to any of Claims 3-9 comprising a dispersant.

11. The detergent composition according to any of Claims 3-10 comprising smectite clays.

(1) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO: 1

```
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
1               5                  10                  15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
            20                  25                  30

Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
        35                  40                  45

Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
    50                  55                  60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
65                  70                  75                  80

Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly
                85                  90                  95

Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
            100                 105                 110

Ala Thr Glu Asn Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn
```

115 120 125

Gln Glu Ile Ser Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
130 135 140

Phe Pro Gly Arg Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr
145 150 155 160

His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg
165 170 175

Ile Tyr Lys Phe Arg Gly Lys Ala Trp Asp Trp Glu Val Asp Ser Glu
180 185 190

Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met Asp His
195 200 205

Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr Thr Asn
210 215 220

Thr Leu Asn Leu Asp Gly Phe Arg Lle Asp Ala Val Lys His Ile Lys
225 230 235 240

Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala Thr Gly
245 250 255

Lys Glu Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu Gly Ala
260 265 270

Leu Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val Phe Asp
275 280 285

Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly Gly Asn
290 295 300

Tyr Asp Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys His Pro

305 310 315 320

Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro Gly Glu
325 330 335

Ser Leu Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala Tyr Ala
340 345 350

Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr Gly Asp
385 360 365

Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala Lys Lle
370 375 380

Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr Gln His
385 390 395 400

Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu Gly Asn
405 410 415

Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp Gly Pro
420 425 430

Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly Gln Val
435 440 445

Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile Asn Ala
450 455 460

Asp Gly Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser Ile Trp
465 470 475 480

Val Lys Arg

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/01615

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/56 C12N9/26 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 26397 A (NOVO NORDISK A/S) 5 October 1995 cited in the application see page 1, line 24 - line 31 see page 2, line 29 - page 3, line 26 see page 12, line 18 - page 30, line 26 ---	3-11
A	WO 96 23874 A (NOVO NORDISK A/S) 8 August 1996 see page 3, line 6 - page 5, line 15 see page 47, line 1 - page 48, line 34 see page 50, line 6 - page 53, line 4 see page 60, line 1 - page 73, line 8 --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1999	22/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/01615

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 09446 A (NOVO NORDISK A/S) 13 March 1997 cited in the application see page 3, line 5 - line 32 see page 4, line 6 - page 6, line 21 see page 7, line 24 - line 36	1,2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/IB 98/01615

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		JP 11500003 T	06-01-1999
WO 9709446 A	13-03-1997	AU 6785496 A	27-03-1997
		CN 1196094 A	14-10-1998
		EP 0854933 A	29-07-1998